Renewable and Sustainable Energy Reviews 37 (2014) 21–35

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Lignocellulosic biobutanol production: Gridlocks and potential remedies

Amruta Morone, R.A. Pandey*

Environmental Biotechnology Division, CSIR-National Environmental Engineering Research Institute (NEERI), Nagpur 440020, India

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form
11 April 2014
Accepted 3 May 2014
Available online 23 May 2014

Keywords:
Biobutanol
Lignocelluloses
Pretreatment
Enzymatic hydrolysis
Butanol tolerance
Process intensification

ABSTRACT

A spike in greenhouse gas emissions due to burning of fossil fuels and issues over energy security and its cost have obligated to identify the alternatives to petroleum fuels currently reigning transportation sector. Butanol, one of the substitutes, is still produced via petrochemical means but the confluence of global issues like declining oil reserves and upsurge in oil prices has compelled to identify renewable biomass resources for butanol production and commercialize the process. Biobutanol is one of the second-generation biofuels, superior to bioethanol, due to higher energy content, lower Reid vapor pressure, easy blending with gasoline at any ratio and ease in transportation. Although bioethanol, a strong competitor of biobutanol, has acquired enough attention from the transportation industry as the current commercially available liquid fuel for transportation, biobutanol possesses the potential to leapfrog various barriers and emerge as an attractive alternative biofuel. Lignocellulosic butanol production faces challenges in various frontiers such as cost of raw material, pretreatment strategies, enzymatic hydrolysis, and low butanol tolerance of the fermenting strain leading to its low yield and productivity, downstream processing of butanol, production of undesired solvents and the production cost. This review discusses these gridlocks along with the possible pertinent solutions to deal with these problems. It also sheds light on recent advancements coupled with the newer approaches for butanol production that revitalize the hopes on having a cleaner, energy-efficient commercial process.

Contents

Abbreviations: LCB, lignocellulosic biomass; LC, lignocelluloses; AFEX, ammonia fiber explosion; WAO, wet air oxidation; GHG, greenhouse gases; ABE, acetone–ethanol–butanol

* Corresponding author. Tel.: +91 712 2240097; fax: +91 712 2249900.
E-mail address: ra_pandey@neeri.res.in (R.A. Pandey).

http://dx.doi.org/10.1016/j.rser.2014.05.009
1364-0321/

1. Introduction

The worldwide energy consumption is increasing and on the other hand, fossil fuels are limited and it is dubious as to how long they will suffice the needs of humankind. The escalating fossil fuel and oil prices and climate change due to greenhouse gases have increased the quest for an alternative fuel that would be economical [1]. Biofuels, which can be produced either chemically or biotechnologically from renewable biomass, are CO_2 neutral and sound to be a promising alternative.

First generation fuels like ethanol, biodiesel and renewable diesel, made from sugars, grains and seeds, are produced commercially in many countries but have led to food vs. fuel war. On the other hand, second generation biofuels, made from non-edible parts of lignocellulosic biomass (hereafter referred to as LCB), are still on the path of commercialization [2]. It is anticipated that these second-generation biofuels will yield better energy, economics and carbon performance than first generation biofuels and are expected to overcome the limitations of first generation biofuels [3].

1-Butanol or n-butanol is a straight chain isomer ending in alcohol functional group. It is considered as superior biofuel as compared to ethanol due to easy blending with gasoline or diesel at any ratio, low octane values, lower energy density, lower Reid vapor pressure, biodegradability and ease in transportation [4–6]. Moreover, LC butanol is favorable from greenhouse gas (GHG) perspective as literature reports indicate 32–48% reduction in GHG due to LC biobutanol as compared to conventional gasoline [7]. Cobalt technologies, a key player in butanol production, claim that Cobalt's process reduces life cycle GHG emissions by 70–90% compared to gasoline [123]. These advantages prove biobutanol as a surrogate for bioethanol, thus, allowing it to take up the mantle.

LC-biobutanol production process involves pretreatment and hydrolysis of raw material followed by fermentation of sugars to butanol. LC-biobutanol is challenging in terms of conversion of lignocellulose to sugars and cost of bioconversion and hence research on commercializing biobutanol still lags behind as compared to that of bioethanol. The principal obstacle is high production cost which includes the capital investment and cost of equipment, raw materials, pretreatment, enzyme, strain development, recovery along with the cost for R&D and sales and marketing of butanol. The process can be made cost-effective by minimizing substrate cost, choosing an efficient pretreatment and hydrolysis method, use of engineered overproducing and butanol tolerant strains for fermentation and simultaneous recovery of butanol. Thus, realization and commercialization of biobutanol from LCB would require technological advancements in the fields of biotechnology and chemical engineering.

Reviews on butanol have been cited in literature reports covering pretreatment, hydrolysis and fermentation separately [8–13]. However, there is scanty information available by integrating all the stages of butanol production together. The novel aspect of the present review is that it covers all the stages of butanol production viz. raw materials, pretreatment, hydrolysis, fermentation, recovery and integration of all the unit operations reflecting the existing status, gridlocks involved at each stage and their remedies. Further, this review also discusses the cost contributing factors and analyzes the R&D strategies and the latest technologies to make the process practical and cost-effective.

Table 1
Substrates used for butanol production.

Sr.№	Substrate	Reference
1	Corn stover	[28]
2	Apple pomace	[29]
3	Cane molasses	[30]
4	Rice, wheat, corn, millet, rye and tapioca	[30]
5	Soy molasses	[31]
6	Corn meal	[32]
7	Wheat straw hydrolysate	[33]
8	Corn fiber hydrolysate	[34]
9	Distillers' dry grain solubles	[35]
10	Barley straw hydrolysate	[36]
11	Corn stover and switchgrass hydrolysate	[37]
12	Sweet sorghum bagasse	[38]
13	Jerusalem artichoke	[39]

2. Raw materials

LCB such as agricultural, forestry, agro-industrial and municipal solid wastes is now catching the eye of many researchers worldwide as an attractive alternative feedstock for butanol production owing to the several benefits it possesses such as high carbohydrate content (cellulose 35–50%, hemicelluloses 20–35%, lignin 10–25%), renewability, abundance, low cost and being carbon neutral [9]. Increased demand for biomass can generate employment in various sectors and provide incentive to improve rural transportation infrastructure, which would facilitate agricultural and economic development. Large scale production of biomass, if made profitable enough can attract farmers to take up the job, ensuring continual supply of biomass and good returns to the farmers, thereby, providing a boost to agribusiness and rural empowerment.

2.1. Bottlenecks

Although LCB is advantageous, several issues such as inconsistency in biomass availability, its composition, land and water requirements, cultivation practices, and logistics costs need to be addressed. These issues are the important factors that contribute to the overall process cost.

Studies by the Ministry of New & Renewable Energy, Government of India, suggest that the biomass availability is 120–150 million metric tons per year [14] including the agricultural and forestry residues. This includes LCB of diverse nature since the quality depends on the crop from which it is derived and its age [15]. Various raw materials used worldwide for butanol production are listed in Table 1. However, availability of a particular biomass may not be constant throughout the year. This poses a severe glitch for the biobutanol production industry.

LCB with high cellulose and hemicellulose content is required for higher yield of butanol. However, it also consists of lignin, ash, protein and waxes in smaller amounts. On one hand where the relative proportions of cellulose: hemicellulose: lignin are the key factors in determining optimum energy conversion route for each type of biomass; the other contents lead to diminution of the theoretical butanol yield (g butanol/g LCB) when compared with sugar and starch crops [16]. This increases the dry weight of LCB required to produce particular volume of ethanol, thereby, escalating the transportation and handling costs. Thus, the best feedstock for a specific product varies depending on the conversion process used, but biomass containing low lignin is always preferable.

Transportation logistics is the key to developing a successful biofuel industry from biomass feedstock and hence, logistics management and careful supply chain planning find a significant place in success of lignocellulosic biobutanol industry [17]. LC-feedstock has considerably lower bulk density, resulting in logistical challenges, which in turn, result in diseconomy. In addition, the cultivation practices in different countries or for that matter several regions in the same country, vary according to the climatic conditions. Thus, the fields providing this LCB are scattered all over the country, thereby, making the collection of raw material difficult. If analyzed closely, the same is true at the global scale too. Moreover, transportation over such long distances is economically unfeasible and adds to the raw material cost [18].

2.2. Potential remedies

A year-round supply of biomass is the foremost requirement of LCB-based biobutanol industry so as to fulfill the daily demand of biomass in industry and to circumvent the biomass storage problems. This problem can be solved by adopting new cultivation methods and crop rotation techniques which might aid in increasing the biomass availability. Moreover, replacement of one biomass residue with another equally competent biomass residue would immensely help in keeping the biomass supply constant round the year for LC-biobutanol production. Alternatively, a successful mélange of two or three different LCBs can also be used to combat the unavailability of biomass throughout the year without intervening in the cultivation practices.

Logistics is the most costly and challenging aspect of the LC butanol industry which further increases exponentially with the plant size. While logistics are often complex, modeling of logistics would be useful in gaining insight into the economic feasibility even before the start of a plant. This calls for optimization of the feedstock logistic system that can integrate pre-processing and transportation of feedstock. The logistics, including the collection, pre-processing and transportation from source locations to the energy plant can be modeled by means of a network structure to simulate the collection, storage, and transport operations for supplying agricultural biomass [19]. Although various models based on optimization and simulation approaches have been modeled and reviewed in literature, a very few of them combine it with a Geographic Information System (GIS)-based approach. For instance, Sokhansanj et al. have developed a dynamic integrated biomass supply analysis and logistics (IBSAL) model using a high level simulation language EXTEND™ to simulate collection, storage and transport operations intended for supplying agricultural biomass to biorefinery [19]. The IBSAL model also accounts for the operational parameters of machines and storage limitations in order to compute LCB supply costs, emissions and energy inputs required [19]. On one hand where it uses the innovative non-linear equations to portray the dependencies of different factors on the supply side, it fails to depict the biomass to bioenergy conversion process and the end uses. This brings in the need for evolution of these models. Another mathematical model presented by Dal-Mas et al. is the mixed-integer linear programming (MILP) model for optimization of corn-based ethanol supply chain in terms of fiscal risk on investments. It deals with the upstream as well as downstream processes involved in bioconversion of biomass to biofuel including the process of biomass cultivation, delivery, fuel production and its distribution to demand centers [20]. Such models would be extremely beneficial if preceded by a GIS-based approach which allows assessing location, transport, storage, processing infrastructure and final energy demand sites by taking into account the transport network, population distribution and preventing co-location of other competitors of biomass feedstock [21]. A further assessment of few other parameters like the tortuosity factor of road infrastructure that takes into consideration the real road network and dispersion of LCB resources with the aid of GIS software will help in accurately designing and determining the LCB delivery cost using real road network [22].

Although land use, existing and competing use of LCB and influence of climate are taken into consideration, some aspects still remain overlooked. These include the sustainability issues such as harvesting, energy inputs in biomass production, direct/indirect land use, infrastructure required for processing steps, logistics, storage and biomass and biofuel transport mode. Therefore, a hybrid approach based on optimization, simulation and GIS might prove to be an effective tool and impart authenticity to the results obtained through these models. The multi-criteria optimization will ease out the selection of potential facility location for LC-biobutanol production on the basis of LCB availability, accessibility and transportation network. Further, it would be immensely helpful in ensuring sustainability and viability of biomass supply chain planning at strategic design level and operational level. Thus, these models will also aid in increasing the efficiency of the entire biofuel supply chain planning of not only biobutanol industry but also other industries using LCB such as the torrefaction operations, LCB co-fired power plants, etc. Ergo, it will be tremendously helpful in concomitantly reducing the cost of feedstock, transportation, energy consumption and GHG emissions.

Energy farming is another means to reduce the cost of raw material by the use of dedicated biofuel crops like switchgrass and *Miscanthus*, which require fallow and marginal lands, have low nutrient requirements, provide high yield and are easy to grow and harvest. This can be advantageous depending on the local climate and soil condition [16]. Alternative raw material especially wastes viz. domestic organic waste, cheese whey, paper waste, etc. may be exploited for butanol production due to the cheaper substrate value [23–25]. Utilization of such kind of waste can considerably solve the problem of waste accumulation, its management and disposal. In addition, this can also economize the butanol production process.

The economic assessment of butanol production based on cellulosic and non-cellulosic feedstock shows that the production data cost for non-commercial biofuels like butanol is less readily available as compared to the commercially available biofuels [26,27].

3. Pre-treatment

The crystallinity and high degree of polymerization of cellulose, its sheathing by lignin and hemicellulose and low accessible surface area, make LCB recalcitrant [40]. Thus, selective removal of cellulose (and subsequently glucose) from this tightly intertwined and complex LC by pretreatment is of utmost importance in the biomass conversion process. This makes pre-treatment of biomass a significant step to make biomass more amenable to subsequent cellulose hydrolysis, thus, reducing the mass transport limitations for chemical or biological catalyst.

Table 2
Comparative evaluation of different pretreatment strategies.

Sr. no.	Pretreatment method	Mechanism of action	Advantages	Limitations	Cost of entire process	Energy	Applicability to wide range of biomass	Technology Status	Remark	Reference
1	Mechanical pretreatment • Chipping • Grinding • Milling	Reduces particle size	Reduces crystallinity	Inability to remove lignin	+	+++	√	Commercial	Processing step for other pretreatment methods	[42]
2	Physical pretreatment • γ-irradiation • Pyrolysis • Microwave irradiation	Use of high temperature leading to cleavage of β-1,4-glycosidic bonds	Reduces crystallinity, increases accessible surface area	Inability to remove lignin, environmental and safety concerns	+++	+++	√/×	Research	Large-scale operation unfeasible	[43]
3	Physicochemical pretreatment									
i	Steam Explosion	Treatment with high-pressure saturated steam by holding it at a high temperature (160–260 °C) and pressure (0.69–4.83 MPa) for a short period (several seconds to few minutes) followed by a rapid decompression	Separation of individual cellulose fibers, alteration of lignin, hemicellulose removal	Generation of inhibitory compounds and degradation of xylan portion	+++	+++	√/×	Pilot-scale	–	[44]
ii	Ammonia fiber explosion	Exposure to liquid ammonia at moderate temperatures (80–150 °C) and pressure (200–400 psi) for 5–30 min followed by instantaneous pressure release	Hemicellulose solubilization, cellulose decrystallization, lignin depolymerization	Ammonia handling and recovery	+++	++	√/×	Research	Unsuitable for biomass containing high lignin	[45]
iii	Liquid hot water	Use of hot water at elevated temperatures (160–200 °C) instead of steam	No additional chemicals and minimum risk of inhibitory product formation	Abundant water usage	+	+	×	Research	Large-scale operation unfeasible	[46]
iv	Supercritical CO_2 explosion	Injection of high pressure CO_2 followed by explosive decompression	Disruption of cellulosic structure and increase in accessible surface area	Not much effect on lignin and hemicellulose	+++	++	×	Research	–	[47]
v	Wet air oxidation	Uses air in combination with water at elevated temperature (125–320 °C) and pressure (0.5–2 MPa) leading to oxidative reaction	Opening of crystalline cellulose, solubilization of hemicellulose and lignin, limited formation of inhibitors	Equipment cost	++	+	√	Research	–	[48,49]
4	Chemical pretreatment									
i	Acid hydrolysis	Use of concentrated or dilute acids	Effective hydrolysis of hemicellulose to xylose and lignin alteration	Neutralization step is necessary, biomass may not be fermentation-compatible, require corrosion-resistant reactors	++	+	√	Pilot-scale trials	–	[50]
ii	Alkaline hydrolysis	Uses lime, NaOH, KOH, $Ca(OH)_2$ to solubilize both lignin and hemicellulose	Effective removal of lignin and acetyl groups of hemicellulose	Neutralization step is necessary, time consuming	++	+	√/×	Pilot-scale trials	Unsuitable for biomass containing high lignin	[51]
iii	Organosolv pretreatment	Uses organic solvents to break lignin-carbohydrate bonds	Separates lignin & hemicellulose	May have inhibitory effect on enzymatic hydrolysis	++	++	√	Pilot-scale trials	Solvents are volatile and hence digester leaks should be avoided	[52]
iv	Ozonolysis	Uses ozone to solubilize and reduce lignin	Reaction at normal temperature and pressure, no formation of toxic compounds	Requirement of large amounts of ozone	+++	+++	√/×	Research	–	[53]

v	Ionic liquids	Ionic liquids compete with lignocellulosic components for hydrogen bonding, thereby, disrupting its three dimensional network	Easy regeneration of cellulose and hemicellulose fibers	Still at research level	++	–	√/×	Research	–	[54]
5	Biological pretreatment	Uses white-, soft- and brown-rot fungi	Toxic and inhibitory compounds are not formed, lignin degradation, high specificity	Very slow	+	+	√/×	Research	–	[55]

+++: Highly significant.
++: Significant.
+: Less significant.

While the effects of pretreatment on the cell wall material are highly complex and still constitute an area of active research, effective pretreatments are known to remove xylan, displace lignin, and de-crystallize the cellulose fibers [10]. Apart from this, it should have simple setup, be cost-effective, energy efficient, limited formation of inhibitors due to sugar and lignin degradation, effective on wide range of LC loading, generate high value lignin co-product in useable form and fermentation-compatible pretreated material.

Pretreatment can either be mechanical, physical, physicochemical, chemical and/or biological. Several pretreatment methods viz. pyrolysis, ammonia fiber expansion (AFEX), steam explosion, wet air oxidation (WAO), alkaline hydrolysis, acid hydrolysis and ozonolysis have been cited in the literature but due to the difference in physicochemical properties of many LCB, the pretreatment method varies in operating conditions for different feedstocks and contributes about 18–20% of the total cost (Table 2) [10,41].

3.1. Bottlenecks

The principal bottlenecks for pretreatment of LCB are the recalcitrance of LCB, use of high temperature and pressure, use of extensive chemicals, abundant water usage, formation of inhibitors and sugar degradation products, energy-intensive and high capital costs.

The pretreatment processes viz. steam explosion [44], AFEX [45], supercritical CO_2 explosion [47], and wet air oxidation [48,49] operate at high temperature and pressure that require high amount of energy, making the process energy-intensive. Moreover, operation at such high temperature and pressure leads to degradation of hexose and pentose sugars to a certain extent. Nevertheless, many of them are unsuitable for biomass containing high lignin content [43,46].

In contrast, chemical methods viz. acid/alkaline hydrolysis, ozonolysis, organosolv pretreatment [44,52], etc. make excessive use of chemicals such as concentrated acids [46,55], oxidizing agents, hydrogen peroxide [56], etc. which necessitate an additional neutralization step to neutralize these chemicals that may interfere in the subsequent hydrolysis and fermentation steps. In addition, these acids are toxic, corrosive and hazardous, which prompt the need for reactors that are resistant to corrosion. This makes the entire process unfeasible [50]. Moreover, acid pretreated materials are sometimes harder to ferment due to the presence of toxic substances [43].

The principal limitation of many pretreatment strategies is partial degradation of pentose sugars and formation of inhibitory and toxic compounds like furan aldehydes and carboxylic acids that may interfere in hydrolysis and fermentation processes [42]. Some of the pretreatments though useful are expensive and may raise environmental concerns, thereby making the process unfeasible at industrial level. In AFEX, cost of ammonia, its handling and recovery, makes the pretreatment more costly [45]. The use of abundant water during the liquid hot water pretreatment makes it economically unfeasible [46]. Wet air oxidation also involves high capital costs [48]. Similarly, ozonolysis, wherein ozone is used to solubilize and reduce lignin, is a costly method due to requirement of large amount of ozone. Thus, the major challenge is to minimize water usage and energy requirements, and limit the excess generation of wastewater and cost of the pretreatment process to make it economically feasible at industrial scale.

3.2. Potential remedies

It is anticipated that a combination of pretreatments might prove to be beneficial since the advantages of both the pretreatments together can yield a potential output and the limitations of one pretreatment may be surmounted by the other pretreatment used in the combination. For example, WAO coupled with alkaline

hydrolysis, combination of biological pretreatments with mild physical or chemical pretreatment sounds to be an effective alternative [57]. This may lead to minimization of the use of chemicals and critical operating conditions viz. high temperature and pressure.

In order to minimize the recalcitrance of biomass, the intervention of plant molecular biology may aid in genetically engineering a plant to contain less lignin in the biomass, thereby, increasing the cellulose: hemicellulose content, which contributes mainly to formation of sugars required for fermentation. Similar efforts on manipulating lignin content have been done in switchgrass cultivars [58,59]. This would probably help in reducing the cost of pretreatment step. However, lignin plays an active role in the defense of the plants and hence removal of lignin may lead to some untoward conditions. Thus, this problem needs to be addressed appropriately.

Application of newer techniques such as ionic liquids (e.g. 1-alkyl-3-methyl-imidazolium cations and various anions) is an interesting weapon in chemist's armory and sounds promising since they are thermostable, non-volatile, environmentally friendly and disrupt the 3-D network of LCB [54]. Another strategy that avoids use of excess chemicals and water is biological pretreatment that uses brown- (mainly attack cellulose), white- and soft-rot fungi (attack both cellulose and lignin) for biological delignification [9,55]. Although biological pretreatment is slow but advantageous in terms of high specificity, low energy requirement for lignin removal, and mild environmental conditions that avoid sugar degradation.

Moreover, for abatement of pretreatment cost, certain strategies can be applied. For instance, dilute acids can replace concentrated acids during the acid hydrolysis since dilute acids (0.4–4%) are inexpensive and effective in hydrolyzing hemicellulose to xylose and other sugars. In addition, 80–90% hemicellulose sugars are recoverable by dilute acid technology [51,56]. The solvents can be removed and recycled in organosolv and ionic liquid pretreatment [52,54]. Bioengineered microbes hold a promise in reducing the cost of pretreatment and can provide solution to the bottlenecks involved in pretreatment strategy. Attempts have been made on in bioengineering of microbes (e.g. *Limnoriaquadri punctata*) for producing the enzymes for lignin degradation [60].

Another strategy that can contribute positively towards the process economics is to utilize the high-calorific value lignin to generate electricity in a power plant that can be integrated with the butanol plant. Nevertheless, the power generated can also be used to supplement the energy requirements of the butanol plant itself, thus making it self-reliant in terms of energy. In addition, lignin can be utilized for obtaining variety of value-added products like vanillin and gallic acid, which are used as anti-foaming and anti-microbial agents, in production of herbicides and as drugs. The pretreatment of hemicellulose results in formation of xylose, which generates xylitol and furfural, which can be put to use as a sweetener and in manufacturing of varnishes and pesticides, respectively [15]. The wastewater generated during the pretreatment step can be anaerobically digested to produce biogas that may be used to feed boilers and power plants.

A scientific and engineering approach is required for selection of appropriate pretreatment strategy and its optimization with respect to particular biomass since the optimized operating conditions of any pretreatment strategy will vary for different biomass owing to the difference in physicochemical properties of biomass and variability in its chemical composition. Additionally, optimization of any pretreatment strategy should also take into consideration the scale-up parameters since the results obtained at bench-scale may not necessarily depict the situation at pilot-scale. Thus, it is suggested that a meticulous scientific outlook is required in order to gain insight into the precise mechanism of the selected pretreatment strategy and an engineering outlook will assist in its proper optimization and will also play a decisive role in determining its scale-up feasibility.

4. Hydrolysis

LCB is high in cellulose and hemicellulose content, which can be converted to soluble sugars by hydrolysis and subsequently fermented to yield butanol. Chemical and enzymatic methods are the commonly employed methods for cellulose and hemicellulose hydrolysis. The chemical method uses high operating temperature and high acid concentration making the process uneconomical; acid neutralization and recovery also involve huge costs [9]. In addition, acid hydrolysis results in poor yield, sugar degradation and generation of wastewater, which warrants further investment for the treatment. Conversely, enzymatic hydrolysis is highly specific, requires low energy, provides higher conversion yields, requires ambient operating conditions, does not lead to corrosion and has lower environmental impact as compared to acid hydrolysis. In addition, there is no generation of toxic inhibitors.

Enzymatic hydrolysis is an eco-friendly process for cellulose conversion to sugars and uses the enzyme cellulase. Cellulases, which may be of fungal or bacterial origin, are a mixture of at least three different enzymes that operate synergistically. This enzyme cocktail includes (i) endo-acting **β-1-4-endoglucanases** (EC 3.2.1.4) which attack the amorphous or low crystallinity regions of cellulose, thus, creating new chain ends for exoglucanases; (ii) exo-acting **β-1-4-exoglucanases** or **cellobiohydrolases** (EC 3.2.1.91) which adsorbs onto the free chain-ends, threads it through its active site and cleaves off the cellobiose units; and (iii) **β-glucosidase** (EC 3.2.1.21) which breaks down cellobiose to produce glucose monomers [15]. Exoglucanases exist in two forms – one that proceeds from reducing end of the chain and other that proceeds from non-reducing end. Hydrolysis of cellulose has been depicted diagrammatically in Fig. 1. A thermodynamic, kinetic, biochemical and molecular simulation might help to gain insight into the mechanism of cellulase action [55,61].

4.1. Bottlenecks in enzymatic hydrolysis of LCB

Complexity and heterogeneity of LCB, which are contributed by cellulose crystallinity, acetyl groups of hemicellulose, and lignin and degree of polymerization, govern the enzyme accessibility, its rate, extent and efficiency [40,62,63]. In addition, limited understanding of basic hydrolysis process also limits the success of cellulose degradation.

The enzyme properties influencing the process of hydrolysis include the enzyme concentration, concoction of enzymes used, its specificity, the enzyme loading and contact time with the substrate. The cost of enzyme is another hurdle in cost-effectiveness of the process since it increases the load on the process economics of LC butanol. This has provoked the hunt for novel high cellulase-producing microbes and development of hypercellulolytic strains using genetic and enzyme/protein engineering. Many cell wall deconstructing enzymes have been isolated and characterized and others are still under investigation [64].

4.2. Potential remedies

A decrease in hydrolysis time, enzyme loading and enzyme cost can be acquired by adopting a proficient pretreatment method and increasing the enzyme efficiency and specific activity since the rate of hydrolysis depends on enzyme loadings, hydrolysis period, and the structural changes occurring during pretreatment.

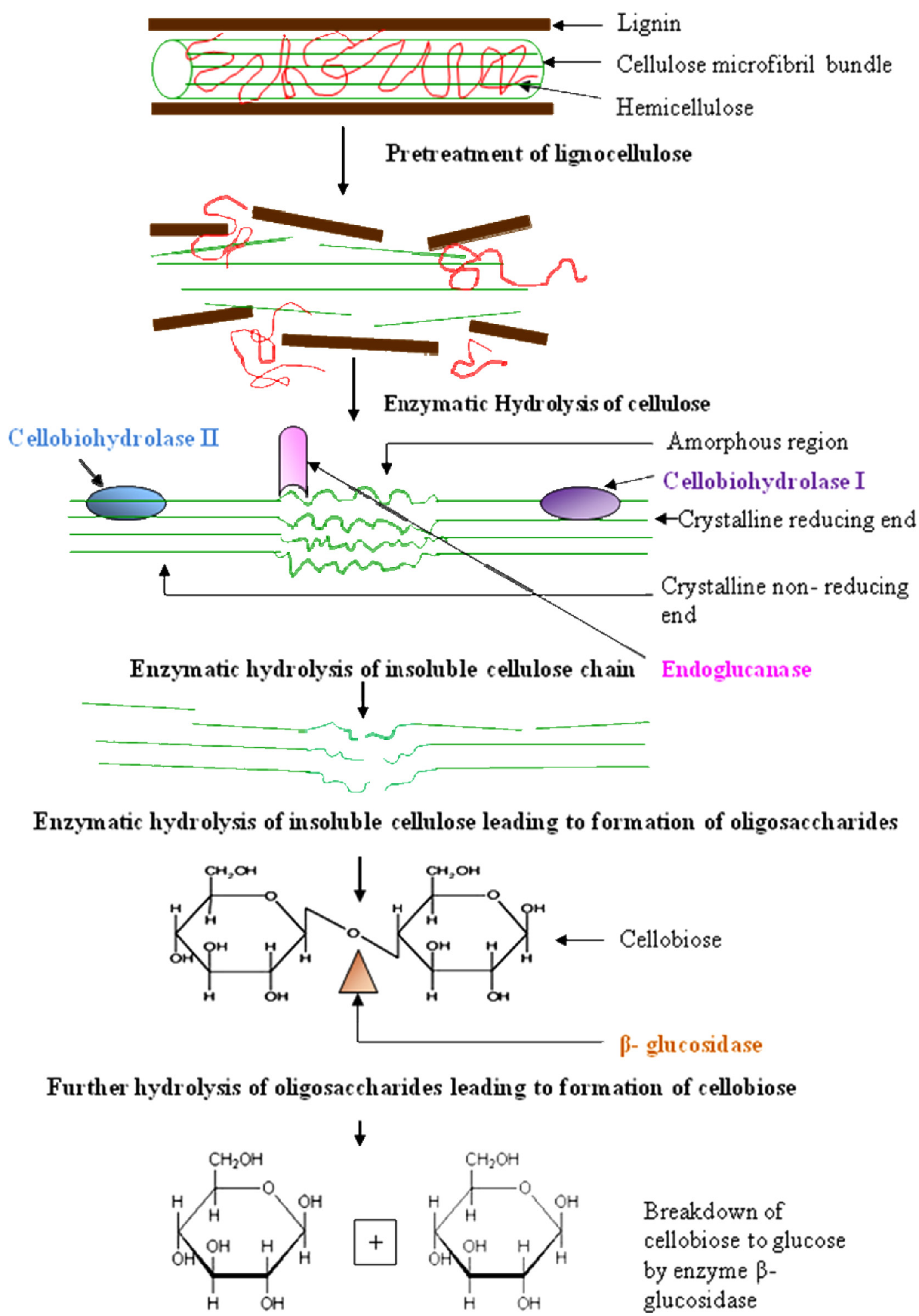


Fig. 1. Schematic representation of enzymatic hydrolysis of lignocelluloses.

An enzyme cocktail consisting of thermostable, highly specific cellulases, β-glucosidases and xylanases (most abundant class of hemicellulases that degrade xylan) in appropriate proportion can help overcome the barriers to cellulose. The ideal cellulase must be highly active on the intended LCB material, must have the ability to completely hydrolyze the biomass and withstand shear forces, operate at mild conditions, and should not inhibit other enzymes in the cocktail [65]. In addition, supplementing the cellulase preparation with the accessory enzymes like xylanase and pectinase can improve the functioning of cellulases. Functional proteins such as expansins and swollenins (obtained from *Trichoderma ressei*) can also promote enzymatic hydrolysis by disrupting the cellulose crystalline structure by breaking the hydrogen bonds between cellulose fibers and polysaccharide polymers [66].

Furthermore, employing the use of additives such as non-ionic surfactants (e.g. Tween 20 or Tween 80) and non-catalytic proteins (e.g. Bovine Serum albumin or polyethylene glycol) might circumvent the glitch of unproductive and irreversible binding of

cellulase to lignin and hemicellulose, which consequently lead to improved enzyme performance. This leads to increase in the enzyme activity level; consequently, enzyme loading is reduced and increase in yield is observed; another strategy to lower the processing costs [55,66].

In order to overcome the biomass-related bottlenecks, ultrasound waves can be applied to the pretreatment and saccharification process, thus, augmenting the rate of enzymatic hydrolysis of pretreated biomass. Zhang et al. have shown that ultrasound assisted with alkali pretreatment enhances the catalytic efficiency by 70% by disrupting the intermolecular hydrogen bonding of lignocellulose and decreasing the crystallinity [67]. The cavitation effect of ultrasound waves and acoustic streaming leads to matrix swelling and partial delignification and intensifies the mass transfer rate [67], thus, enhancing the transport of enzyme molecules towards the substrate surface. Moreover, the mechanical impacts produced by collapse of cavitation bubbles assist in opening up the surface of solid substrates for the enzyme action [68]. Studies on the effect of ultrasonic intensity and reactor scale on the kinetics of enzymatic saccharification depicts an efficient hydrolysis on increasing the specific ultrasonic intensity [69].

In addition, enzymatic hydrolysis at high solid loading and low enzyme loading demonstrates the production of high sugar yield [70]. Another problem is the deactivation of enzyme, which increases the enzyme loading. The feedback inhibition of cellulases occurs due to the inhibition of cellulose binding domain by high concentration of the products (e.g. cellobiose and glucose). Simultaneous saccharification and fermentation may provide a key to the problem of deactivation of enzyme. On-site enzyme production, improvement in enzyme specific activity and recycling or recovering the enzyme by ultrafiltration, ion exchange chromatography and immobilization can further aid in reducing enzyme cost [55]. Some approaches such as employing membrane for enzyme separation for its reuse and fed-batch strategy over batch mode hydrolysis might also prove effective in order to increase the sugar yield at high biomass loading and low enzyme loading.

The researchers need to focus on developing the cost-effective enzyme system suitable for effective enzymatic hydrolysis of LCB. Moreover, the left-over fraction of LCB after hydrolysis has a high tensile strength and can be explored for generation of various value-added products. Additional research on the molecular mechanism and optimization of hydrolysis process and reaction conditions may help in understanding and overcoming the gridlocks involved in the process and would provide a breakthrough in butanol production.

4.3. Current market scenario of commercial cellulase enzymes

Enzyme manufacturing giants such as Genencor International, Novozymes Inc., are the current international players in commercial production of cellulases. The launch of Accelerase® 1500, a cellulase complex intended specifically for LCB, by Genencor has significantly improved the formulation and activity. Genencor has also launched Accelerase® XY, an accessory xylanase enzyme and Accelerase® BG, an accessory β-glucosidase, which when blended with cellulase can prove to be highly efficient [71]. On similar lines, Novozymes have unveiled its latest innovation, NovozymesCellic® CTec3, on 22 February, 2012 [72]. Other companies such as Amano Enzyme Inc. in Japan, Dyadic are also not lagging behind in the race to produce a cutting edge enzyme and have launched their enzyme products – Viscostar 150 L (Dyadic, USA), Cellulase AP30K and Cellulase TAP106 (Amano Enzyme Inc., Japan). Recent launch of CodeXyme™ has marked the entry of Codexis' into global cellulase market [73].

5. Fermentation

Traditionally, butanol was produced by acetone–butanol–ethanol (ABE) fermentation process in the ratio 3:6:1 using *Clostridium acetobutylicum* as the fermenting strain [74]. ABE fermentation involves two sequential phases – the first phase comprises of 'acidogenesis' phase wherein an exponential growth phase is observed along with conversion of glucose to butyric acid while in the second phase a fermentation switch is encountered and butyric acid is converted to butanol via 'solventogenesis' phase [75]. Currently, butanol is being produced via petrochemical route, which is not an environmentally conscious method of making butanol. Ergo, it becomes essential to eliminate the barriers in fermentation in order to maximize butanol productivity.

A variety of different process configurations are available for fermentation of pretreated and hydrolyzed biomass. Fermentation configuration available for LC-biobutanol production includes separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP) [77–80]. All these fermentation configurations can be operated in batch, fed-batch and continuous modes with their own pros and cons. However, butanol productivity in batch mode is lower than that in fed-batch and continuous mode [76,81,82].

Consolidated bioprocessing (CBP) is deemed as a promising approach for biobutanol production since it uses cellulolytic microorganisms that produce cellulase and perform hydrolysis and fermentation in a single step. *Clostridium thermocellum*, *Clostridium sterocorarium* and *Bacteroides cellulosolvens* have been investigated for high efficiency CBP and have been used for isobutanol production through direct cellulose use from *Clostridium cellulolyticum* [79,80].

A two-stage fermentation approach can be adopted wherein in first stage, *Clostridium tyrobutyricum* ferments biomass to butyric acid while in second stage, *C. acetobutylicum* ferments butyric acid to butanol [83]. Increase in efficiency of butyric acid production was observed by the immobilization of *C. tyrobutyricum* onto a packed bed of fibrous matrix in a fibrous bed bioreactor and a fluidized bed reactor [39,84].

5.1. Bottlenecks

The bottlenecks in ABE fermentation include low butanol titers that reduce sugar loadings, production of unwanted solvents, low butanol tolerance by fermenting strain and low productivity. Collectively, these gridlocks result in significant increase in the entire process cost.

In a typical batch process, total solvent concentration generated by ABE fermentation amounts to 20 g/L, of which, butanol accounts to just 13 g/L. This low butanol yield and productivity is due to low cell densities that are caused by lack of butanol tolerance by the fermenting strain [11]. Another gridlock encountered during fermentation is sporulation of the fermenting strain. Sporulation and solventogenesis are contemporaneous; though, sporulation is not essential for solvent formation since sporulation directs the bacterial cells to enter a state of dormancy wherein they do not produce solvents [85,86]. A key regulator of solventogenesis, Spo0A, which on phosphorylation begins sporulation, has been identified in conjunction with the sigma factors/transcription regulators (σ^E, σ^F, σ^G, σ^K), whose inactivation may lead to unyoking of sporulation and solventogenesis [87].

5.2. Potential remedies

Attempts have been made to increase the butanol titer by constructing a customized clostridial butanol pathway in *Escherichia coli*. In this attempt, the driving forces NADH and acetyl-CoA

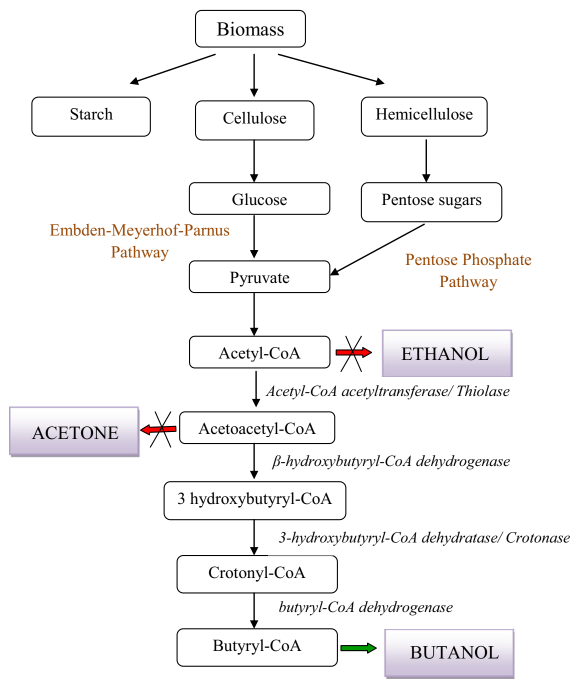


Fig. 2. Butanol production pathway and approaches to increase butanol production. The electron donors and acceptors are not shown here for avoiding complexity.

lead to production of butanol in high titer of 30 g/L and high yield (70–88% of the theoretical) [88].

As stated earlier, acetone and ethanol are also formed along with butanol during ABE fermentation. This production of unwanted solvents is a burden, which influences the cost of ABE fermentation. This calls for engineering of the process and/or solventogenic clostridia in order to increase butanol production and suppress the production of unwanted solvents, thereby, plummeting the cost of overall fermentation process. In order to redirect the carbon flow from ethanol and acetone towards butanol, deeper insight and profound knowledge of the regulation of metabolic flux are obligatory [87].

The biosynthesis of acetone, ethanol and butanol occurs by a central pathway that later bifurcates to produce the respective solvents (Fig. 2). Acetyl-CoA, which is involved in butyryl-CoA formation, is the principal branch point for these different pathways. However, during the above conversion process, acetyl-CoA condenses to form acetoacetyl-CoA, which is the rate-limiting step since it is thermodynamically unfavorable with the standard Gibbs free-energy change of 5.3. In order to make this reaction thermodynamically favorable, a high concentration of acetyl-CoA is required [86]. This may be done by overexpression of the gene involved in acetyl-CoA production. In order to inhibit production of unwanted solvents, the existing side pathways can be blocked so that carbon and electron flow are directed towards butanol. This can be done by inhibiting the enzymes involved in side pathways, thereby preventing formation of byproducts. Thus, selectively producing butanol may ease out the recovery process, too. Some efforts on utilization of antisense RNA technology for downregulation of a few genes encoding enzymes involved in butanol synthesis have been reported [89].

The most important bottleneck in selection of strain for LC butanol production is butanol tolerance capacity of the fermenting strain. It is reported that the cell metabolism usually ceases after the solvent reaches the inhibitory levels of around 2% (v/v), thereby, demonstrating their inability to surmount the 2% butanol barrier [90]. The toxicity of butanol to the fermenting strain is measured in terms of partition coefficient of butanol, represented by log *P* value [91]. Butanol has a lower log *P* value of 0.8, which indicates greater polarity of the solvent, thus exposing the cells to high concentration of solvent and thereby, mediating a toxic response [92]. The mechanisms for butanol tolerance by the fermenting strain include membrane modifications like membrane adaptation by increasing the membrane rigidity and declining membrane permeability by *cis–trans* isomerization.

Although many microorganisms including *E. coli* [93], *Pseudomonas putida* [92], *Bacillus subtilis* [94], *Lactobacillus brevis*, *Lactobacillus delbrueckii* [95] and many others have been exploited for engineering butanol pathway and increasing butanol tolerance, *E. coli* is still the scientific paradigm for genetic manipulation. This is due to the wealth of knowledge about this microorganism being well documented and all its genes have been identified along with a vast majority of its pathways [96]. Atsumi et al. have demonstrated the production of butanol by engineering a synthetic butanol-producing pathway in *E. coli* [93]. A computational approach can aid in designing and analyzing new and innovative pathways to enhance butanol production. The attractive pathways can be selected based on the pathway length, metabolic flux analysis, molecular docking, and thermodynamic feasibility [97]. Ranganathan and Maranas, through *in silico engineering* have suggested new pathways and genetic interventions for overproduction of 1-butanol using *E. coli* [98].

The synthetic biology approach wherein alteration in gene regulation is espoused to inspect and exploit bacteria for enhancing butanol yield and butanol tolerance can also be adopted. This can be done by a complex approach of targeting more than one gene simultaneously or by engineering robustness in the bacteria in order to produce desired solvents and maintain maximum carbon flux towards it regardless of changes in physiological conditions [99,100]. Butanol tolerance can be significantly increased by changes in composition of membrane proteins and lipids, membrane hydrophobicity, engineering efflux mechanisms, and overexpression of heat shock proteins since these are upregulated in response to solvent stress [86,91,95,101,102].

6. Butanol recovery

Butanol recovery from the fermentation broth involves capture of butanol from the aqueous stream followed by its purification and recycling of the aqueous stream back to the fermentor. Butanol can be either in vapor, liquid, solid, or supercritical phase after its separation from the aqueous stream, thereby, indicating that butanol separation is regulated by its phase behavior. The separation can be energy-based separation, mass-based separation and kinetically based separation [103]. Gas stripping, reverse osmosis, perstraction, liquid–liquid extraction, vacuum stripping, membrane distillation, vacuum membrane distillation and pervaporation are the eminent recovery processes employed for in situ selective separation and simultaneous removal of butanol and are presented in details in Table 3, citing their advantages and limitations along with a comparative evaluation of energy requirements [76,104–106]. The schematic representation of these processes is given in Fig. 3a–d.

6.1. Bottlenecks

The low butanol concentration in the fermentation broth results in a dilute product stream making the process energy-intensive and pricey. Incorporating a simultaneous or online/in situ removal of butanol from the fermentation broth provides an appropriate solution to prevent the inhibition by the solvents and improvement in solvent productivity [107].

Phase transition, an important aspect in butanol recovery, can occur from liquid to vapor or solid phase. The enthalpy of transition

Table 3
Comparative evaluation of butanol recovery process.

Sr. no.	Recovery method	Advantages	Limitations	Efficiency	Operating cost	Energy requirement (kcal/kg butanol)	Technology status	Reference
1	Distillation	Simple, well-established conventional process	High operating cost, intense energy consumption and azeotrope formation	++	+++	5789	Commercial but cannot be applied for butanol recovery	[76,103,109]
2	Gas stripping	Simple, prevents fouling and clogging, use of external unit allows heating without disturbing temperature of fermentation broth	High operating cost	++	+++	5220	Research	[104,112–114]
3	Solvent Extraction	Easy extraction and recovery of extractant, extremely non-polar extractant leads to high selectivity	Extractant toxicity, rag layer formation	+++	++	2119	Research	[76,115]
4	Pervaporation	Selective removal of butanol, membrane stability	Fouling, high cost	+++	+++	3295	Research	[76,103,104]
5	Adsorption	Energy-efficient recovery	Low capacity of adsorbent, unsuitable for industrial scale	+++	+	1948	Research	[109]
6	Perstraction	Avoids direct contact of extractant and fermentation broth	Fouling, high cost	+++	+++	NA	Research	[104,109]
7	Reverse osmosis	Simple	Solubility of R.O. membranes in acetone, which is one of the byproducts	+	++	NA	Not applied for butanol recovery	–
8	Liquid–liquid demixing	Employs salts to reduce the solubility of butanol and phase separation	Salt concentration is too high and may disturb cells' internal environment	+	++	NA	Research	[103]
9	Freeze crystallization	Thermodynamically favorable due to lower enthalpy of fusion than enthalpy of vaporization of butanol and water	Additional cost of solids handling	++	++	NA	Research	[108]
10	Ionic liquids	Non-volatile, environmentally friendly	Non-toxicity of the ionic solvents should be verified before application	++	++	NA	Research	[105]

+++: Highly significant.
++: Significant.
+: Less significant.

from liquid to vapor (ΔH_{vap}) of butanol is 43.29 kJ/mol while that for water is 40.65 kJ/mol. Moreover, enthalpy of fusion (ΔH_{fus}) of butanol is 9.37 kJ/mol while that for water is 6.01 kJ/mol [108]. Butanol has slightly higher value of enthalpy when calculated on molar basis while enthalpy of water is 3 times higher than butanol when calculated based on mass [108]. This unfavorable thermodynamics of butanol–water system make the recovery of butanol difficult through distillation. Moreover, ABE fermentation process is a multi-solvent system, making separation of butanol even more complicated thermodynamically.

Energy consumption during the recovery process has a significant contribution towards the cost. The butanol capture step alone contributes to about 90% of the energy consumption when butanol is concentrated at 50%. Nevertheless, most of the systems do not achieve a 50% concentration. The energy requirement is estimated based on the enthalpy change of the product portion of the feed flow and is a function of temperature and pressure [109].

6.2. Potential remedies

The primary bottleneck in the butanol recovery process i.e. the dilute product stream can be surmounted by increasing butanol titer using the cell immobilization technique (discussed in Section 5) and a membrane reactor. An improved membrane cell recycle reactor system provides a robust strategy to increase cell concentration by returning the cells to the bioreactor using a membrane filter and clear liquid containing butanol and other solvents are removed for further separation [110]. This increases the reactor productivity and eases the recovery process. Tashiro et al. have reported to have obtained a high ABE production in continuous culture with high cell density by cell recycling and cell bleeding without strain degeneration [110]. However, the principal drawback of this technique is membrane fouling which can be overcomed through module design [106].

The microreactor system for extraction process is also an attractive alternative having small volume and allowing better reaction control due to laminar flow operation [111]. The liquid–liquid microreactor system employs phase transfer catalysis wherein catalysts penetrate the interface between two immiscible liquid phases. For instance, the liquid–liquid microreactor system has been employed for extraction of butanol from toluene [111]. The same technology can be applied for butanol recovery from fermentation broth.

Application of single recovery process may not serve the purpose since every recovery process mentioned in Table 3 has its own advantages and disadvantages. Thus, it is suggested to have a hybrid process – a combination of one or more recovery processes at different stages of fermentation. This will bring flexibility in the separation process that can be carried out at any stage. Consequently, multi-stage recovery can lead to recovery of not only butanol but also many fermentation intermediates that can be further used for different purposes.

7. Process intensification

Process intensification, a new concept that involves developing a considerably cleaner, smaller and energy-efficient technology, is

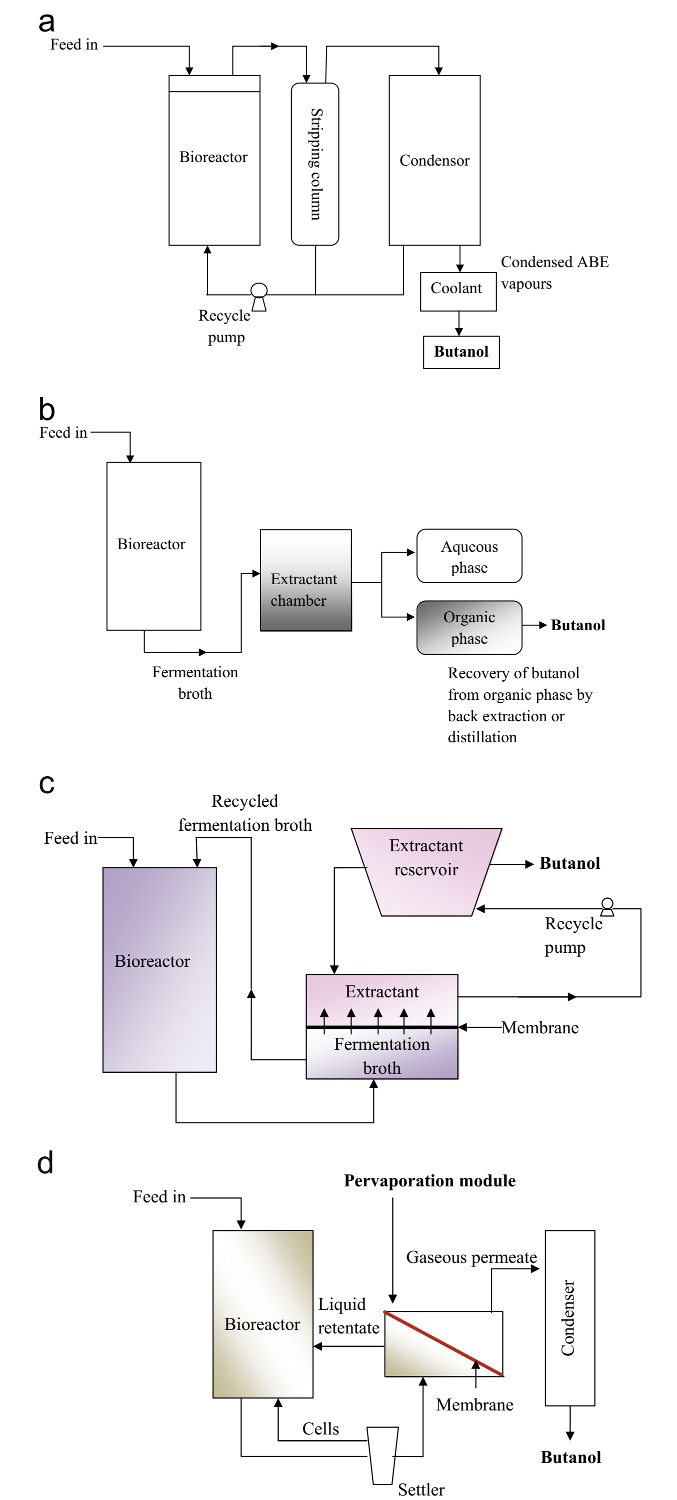


Fig. 3. (a) Gas stripping as a technique for butanol recovery. (b) Liquid–liquid extraction. (c) Perstraction – a technique for butanol recovery. (d) Pervaporation as a means of butanol recovery.

catching the eye of process engineers and has spurred an interest among them for designing new plants and modifying the existing ones with proper integration of the unit operations so that the overall process becomes techno-economically viable [111]. This may help in designing novel process configuration and a simplified system with increased reaction efficiency. The process intensification can be achieved either through unit-based or plant-based intensification [111,116].

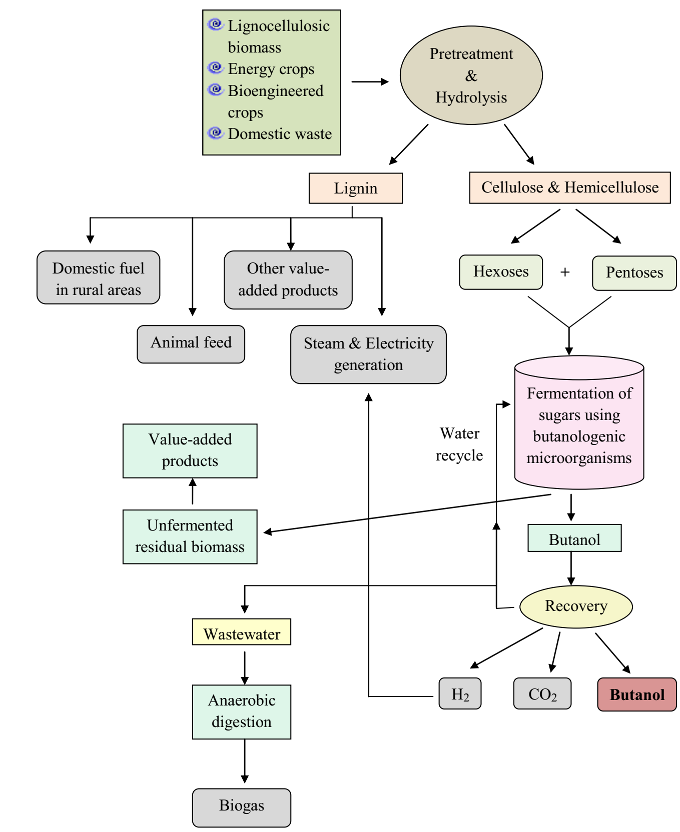


Fig. 4. Process integration and business opportunities to increase process economy during butanol production from lignocellulosic biomass.

The possible approaches for process integration can be integrating all stages together, integrating hydrolysis stage with fermentation, or integrating hydrolysis, fermentation and recovery stages together. In one such attempt, simultaneous saccharification and fermentation were integrated in an oscillatory baffled reactor, wherein a better mixing was observed leading to increased glucose yields [117]. Similarly, the possible integration in butanol production and business opportunities involved in the same have been projected and presented in Fig. 4. However, this integration may lead to some bottlenecks in the existing butanol production process.

7.1. Bottlenecks

Biobutanol production can be analyzed in order to maximize the production through appropriate process integration for all the unit operations. This may lead to minimum input of raw material, energy, fresh water and manpower with high output of butanol and low amount of wastewater. The main bottlenecks involved in process intensification involve variability in utilities, unit operations and waste management.

Butanol production process involves different operating conditions (temperature, pH, etc.) at each step of the process. For instance, the pretreatment of the biomass occurs at high temperatures (120–200 °C) while the enzymatic hydrolysis occurs at a temperature of 40–50 °C and fermentation is done at 35–37 °C. Moreover, the solid loadings and their handling at each stage of the process including raw material loading during pretreatment and hydrolysis are different which may require complex modeling and extensive efforts in equipment designing and may increase the operational complexity. This would further increase the scale-up risks and in turn, the development costs.

Table 4
Comparative evaluation and a sneak peek into current butanol industries.

Sr. no.	Butanol industry	Raw material	Microorganism	(Expected) yield	Approach	Reference
1	Butamax™ Advanced Technologies	Corn	–	–	–	[120]
2	Gevo®	Corn, sugar and beets	Modified strain of *E. coli*	1 Million gallon butanol per year	GIFT® (Gevo's Integrated Fermentation Technology®)	[121]
3	Cathay Industrial Biotech	Corn	–	21 Million gallons of biobutanol per year	Anaerobic fermentation	[122]
4	Cobalt Technologies	Non-food feedstock	Non-GMO *Clostridium*	1.5 million GPY	–	[123]
5	Green Biologics Ltd. (formerly Butyl fuel LLC)	Sugar, starch, molasses, corn byproducts and cellulosic feedstocks	Solventogenic *Clostridium*	1.3–1.9 Times more butanol per weight of raw material	ABE fermentation; metabolic engineering of *Clostridium* and *Geobacillus*	[124]
6	SyntecBiofuel	MSW, forestry and agricultural waste	-	110 gal/t	Catalyst development for converting cellulosic biomass to butanol using thermochemical process	[125]
7	Butalco	C5/C6 sugars and lignocellulose	Genetically optimized yeast	–	Improving existing industrial production strains	[126]

Since LC-butanol production is a multi-solvent system, all the three solvents have different thermodynamic properties and thus, require strategic separation of these by-products. Process intensification should also aim at minimizing these by-products and employing an appropriate product recovery method for increasing the efficiency of the plant.

7.2. Potential remedies

LC-butanol production can be made economical and cost effective only when all the process steps are efficiently integrated. Another approach can be the modification of an existing operational ethanol plant to butanol plant so that some existing ethanol plant equipments can be integrated into the butanol plant, thereby, decreasing the investment.

Waste management and handling is a critical issue in process integration. In order to minimize the water requirements, the wastewater stream generated at each process step may be treated and recycled and reused to make the plant a zero-discharge plant. Similarly, the excess heat generated during pretreatment can be utilized for maintaining the temperature of hydrolysis and fermentation units. Butanol production results in generation of gases like H_2 and CO_2 wherein hydrogen gas can again be put to use for generating electricity while CO_2 gas can be harnessed for butanol production due to the ability of *Clostridium ljungdahlii* to utilize CO_2/H_2 [118].

Thus biobutanol plant can be integrated with biohydrogen and biomethane plant too, which would stupendously lead to an increment in additional revenue generation. An appropriate process integration of all the unit operations involved in biomass conversion process is an area where an incremental and sweeping innovation is prophesied to provide major benefits and make the entire process a money-spinner by exploring the value-added by-products produced in each stage of the process.

8. Current scenario of lignocellulosic butanol industry

Commercializing butanol production in present-state economy sounds expensive. Scaling up from a laboratory scale to pre-industrial scale has always been challenging. This can be explicitly due to less reproducibility of the pilot plant trials, which further increases the degree of uncertainty involved in scale-up process. Sometimes the results obtained are kept confidential due to the commercial interests and when a process has to be patented. Nimcevic and Gapes have reviewed many acetone–butanol plants that have been operational a few years back [119]. Cobalt Technologies, Gevo Inc., Green Biologics Ltd., Butyl Fuel LLC, TetraVitae Bioscience, Plantaonix, Butamax (DuPont/BP), Cathay Industrial Biotech, ButalcoGmBH, Metabolic Explorer and W2 Energy are the key players in butanol production.

The comparative status of these commercial players has been compiled and presented in Table 4. The raw material used for butanol production by these industries ranges from sugars to lignocellulosic biomass. Most of these industries use the conventional fermentation technique using the natural biobutanol producer *Clostridium* while others have ventured into genetic engineering of different strains. In some cases, the yields are expected to range from 1.5 to 21 million gallons of biobutanol per year using various raw materials while in other cases the data is not disclosed due to commercial reasons. A critical analysis of this data indicates that the raw material, microorganisms and fermentation process are the key factors governing the extent of butanol production. However, there are no significant details available in open domain with reference to pretreatment strategies and the enzyme cocktail employed by these commercially operating plants. In addition, the details with reference to downstream processing of the fermented broth are also kept under wraps. These developments with reference to the commercial production of biobutanol is only limited to developed countries except China. However, the developing countries lag behind with respect to commercialization of biobutanol as most of the LCB is diverted as feed for cattle or used as heat energy at household level. Nevertheless, biobutanol commercialization is still under research phase in developing countries and initiatives have been taken to promote R&D in this arena of biofuels.

9. Concluding remarks and future prospects

Energy and environmental concerns have revived an interest in renewable resources. LCB is anticipated to be the biomass of future for butanol production since it is available in surplus amounts and is not only environmentally benign but economically sound too. Despite the immense information on different aspects of butanol production, the commercialization of this process has met with a limited success. Therefore, the technology gridlocks in LC butanol production have to be surmounted in order to envision a better future of LC butanol production. The scientific and technological advances in biological sciences and bioprocess engineering are

supposed to provide additional momentum to take butanol production from its budding phase to a flowering phase. Therefore, to bring this biofuel at its zenith, the factors influencing the process economics and efficient commercialization have to be tackled. This review has endeavored to elucidate all the gridlocks and project the potential remedies for the same which include setting up of the butanol plant in propinquity to the point of raw material production, selecting an appropriate pretreatment technique and enzyme system, employing solvent-tolerant and hyper-butanologenic fermenting strain, on-line butanol recovery and implementation of biorefinery concept.

Although a great deal of research is carried out in this arena since a few years, some gridlocks still persist and need to be dealt with in order to accomplish the success of LC-butanol commercialization. A prudent R&D strategy is still required for a systematic design of a feedstock transportation system as logistics remain a principal gridlock in the LCB bioconversion to butanol even now. The future R&D should focus on development and evolution of mathematical models through advanced computational tools to improve the complex supply chain planning and management while taking into consideration the standard rules and regulations for calculating the transportation cost and performance-based equipment configuration. Further, geographical location of the local, regional and global networking with reference to supply of raw material has to be accurately incorporated as per the existing logistics in order to develop a real supply chain model. A systematic, strategic design and planning of biomass and biofuel supply chain network are suggested in order to meet the daily demand of LCB throughout the year. It is recommended to not only consider the biomass-to-biofuel supply chain but also incorporate biofuel-to-bioenergy supply chain during logistics modeling.

Further, an acute analysis of lignin chemistry should be done since it is an integral part of any pretreatment strategy and would also play a decisive role in its selection and optimization. This may aid in gaining a significant insight into the effectiveness of a particular pretreatment strategy. In addition, future R&D should focus on ascertaining the scale-up feasibility of the selected pretreatment by enhancing the reproducibility of the bench-scale studies. Appropriate integration of the pretreatment process with rest of the process is also of immense importance. The researchers need to direct their efforts on developing a cost-effective enzyme cocktail suitable for effective enzymatic hydrolysis of the LCB and also attempt to increase the sugar concentration at high solid loading and low enzyme loading through different approaches like the fed-batch operation of enzymatic hydrolysis.

The upcoming research work in the fermentation step should be aimed at increasing the butanol yield and tolerance by employing the advanced tools in biotechnology and/or bioprocess engineering. Genomics revolution, modulation of gene expression, protein engineering, rational and combinatorial engineering of bacterial cells and their metabolic pathways will help in reprogramming these bacterial little robots and lo, these microbial chemical factories will start producing increased amounts of butanol. The chemo-biological approach for butanol production, wherein sugars will be converted to butyric acid via biological means in first stage followed by chemical conversion of butyric acid to butanol in second stage, is suggested to be a promising approach. These approaches can be complemented with the on-line removal of butanol, thereby reducing its contact with the fermenting strain so as to enhance its tolerance to butanol. An efficient integration of all the process steps along with reduction in waste generation and water requirements will further foster the success of LC butanol plant. Besides, much more R&D intervention is required to develop some of the novel chemicals and by-products from the residual biomass and fermentation process. The commercial exploitation of these by-products that are formed or can be produced during the entire process would lend a hand in increasing market incursion of butanol. Succinctly summing up, bioscience and bioengineering together can put a step forward towards successful industrial butanol production.

Acknowledgements

The authors acknowledge the suggestions of the reviewers which have helped greatly in improving the quality of manuscript.

References

[1] Biobutanol: the next biofuel. Techno-economic and market evaluation by Nexant and Chemical Strategies; 2009.
[2] Kopke Michael, Noack Steffi, Dürre Peter. 2011, In: Marco Aurélio dos Santos Bernardes (Ed.) The past, present, and future of biofuels –biobutanol as promising alternative. Biofuel production – recent developments and prospects. p. 451–86.
[3] Antizar-Ladislao B, Turrion-Gomez JL. Second-generation biofuels and local bioenergy systems. Biofuels, Bioprod Biorefining 2008;2:455–69.
[4] Arnold F. The race for new biofuels. Eng Sci 2008;71(2):12–9.
[5] Szulczyk K. Which is a better transportation fuel-butanol or ethanol? Int J Energy Environ 2010;1(3):501–12.
[6] Cascone R. Biobutanol – a replacement for bioethanol? Nexant, Inc. in society of biological engineering "Biofuels" special section in the AIChE magazine Chem Eng Prog 2008, vol. 104, S4-S9.
[7] Wu M, Wang M, Liu J, Huo H. Assessment of potential life-cycle energy and greenhouse gas emission effects from using corn-based butanol as a transportation fuel. Biotechnol Prog 2008;24:1204–14.
[8] Schubert C. Can biofuels finally take center stage? Nat Biotechnol 2006;24:777–84.
[9] Mussatto SI, Teixeira JA. Lignocellulose as raw material in fermentation processes. In: Mendez-Vilas A, editor. Current research, technology and education topics in applied microbiology and microbial biotechnology. Formatex; 2010. p. 897–907, Badajoz, Spain.
[10] Mosier N, Wyman C, Dale B, Richard E, Lee Y, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 2005;96:673–86.
[11] Tashiro Y, Sonomoto K. Advances in butanol production by clostridia. In: Mendez-Vilas A, editor. Current research, technology and education topics in applied microbiology and microbial biotechnology. Formatex; 2010. p. 1383–94, Badajoz, Spain.
[12] Ezeji T, Blaschek H. Butanol production from lignocellulosic biomass. In: Blaschek HP, Ezeji TC, Scheffran J, editors. Biofuels from agricultural wastes and byproducts. Blackwell Publishing, United States of America; 2010. p. 19–37.
[13] Dellomonaco C, Fava F, Gonzalez R. The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb Cell Fact 2010;9:3.
[14] Biomass power and cogeneration programme. 〈http://www.mnre.gov.in/related-links/grid-connected/biomass-powercogen/〉.
[15] Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2003;2(12):602–19.
[16] McKendry P. Energy production from biomass (Part 1): overview of biomass. Bioresour Technol 2002;83:37–46.
[17] Rentizelas A, Tolis A, Tatsiopoulos I. Logistics issues of biomass: the storage problem and the multi-biomass supply chain. Renew Sust Energ Rev. 2009;13(4):887–94.
[18] Richard TL. Challenges in scaling up biofuels infrastructure. Sci Mag 2010;329:793–6.
[19] Sokansanj S, Kumar A, Turhollow AF. Development and implementation of integrated biomass supply analysis and logistics model (IBSAL). Biomass Bioenergy 2006;30(10):838–47.
[20] Dal-Mas M, Giarola S, Zamboni A, Bezzo F. Strategic design and investment capacity planning of the ethanol supply chain under price uncertainty. Biomass Bioenergy 2011;35(5):2059–71.
[21] Zhang F, Johnson D, Sutherland J. A GIS-based method for identifying the optimal location for a facility to convert forest biomass to biofuel. Biomass Bioenergy 2011;35:3951–61.
[22] Sultana A, Kumar A. Development of tortuosity factor for assessment of lignocellulosic biomass delivery cost to a biorefinery. Appl Energy 2014;119:288–95.
[23] Claassen P, Budde M, López-Contreras A. Acetone, butanol and ethanol production from domestic organic waste by solventogenic clostridia. J Mol Microbiol Biotechnol 2000;2(1):39–44.
[24] Foda M, Joun H, Li Y. Study the suitability of cheese whey for bio-butanol production by clostridia. J Am Sci 2010;6(8):39–46.
[25] Ray K.H. Cars could run on recycled newspaper. Tulane scientists say. 〈http://tulane.edu/news/releases/pr_082511.cfm〉; 2011.

[26] Kumar M, Goyal Y, Sarkar A, Gayen K. Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. Appl Energy 2012;93:193–204.
[27] Tao L, Aden A. The economics of current and future biofuels. in vitro Cell Dev Biol: Plant 2009;45:199–217.
[28] Lloyd A. French push ahead with biomass conversion. New Sci 1984;102:21.
[29] Voget C, Mignone C, Ertola R. Butanol production from apple pomace. Biotechnol Lett 1985;7(1):43–6.
[30] Jones D, Woods D. Acetone–butanol fermentation revisited. Microbiol Rev 1986;50:484–524.
[31] Qureshi N, Lolas A, Blaschek H. Soy molasses as fermentation substrate for production of butanol using *Clostridium beijerinckii* BA101. J Ind Microbiol Biotechnol 2001;26:290–5.
[32] Huang YL, Wu Z, Zhang L, Cheuang CM, Yang ST. Production of carboxylic acids from hydrolyzed corn meal by immobilized cell fermentation in a fibrous-bed bioreactor. Bioresour Technol 2002;82:51–9.
[33] Qureshi N, Saha B, Cotta M. Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*. Bioprocess Biosyst Eng 2007;30:419–27.
[34] Qureshi N, Ezeji T, Ebener J, Dien B, Cotta M, Blaschek H. Butanol production by *Clostridium beijerinckii*. Part I: use of acid and enzyme hydrolyzed corn fiber. Bioresour Technol 2008;99:5915–22.
[35] Ezeji T, Blaschek H. Fermentation of dried distiller's grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia. Bioresour Technol 2008;99:5232–42.
[36] Qureshi N, Saha B, Dien B, Hector R, Cotta M. Production of butanol (a biofuel) from agricultural residues: Part I – use of barley straw hydrolysate. Biomass Bioenergy 2010;34(4):559–65.
[37] Qureshi N, Saha B, Hector R, Dien B, Hughes S, Liu S, et al. Production of butanol (a biofuel) from agricultural residues: Part II – use of corn stover and switchgrasshydrolysates. Biomass Bioenergy 2010;34(4):566–71.
[38] Zhang T, Du N, Tan T. Biobutanol production from sweet sorghum bagasse. J Biobased Mater Bioenergy 2011;5(3):331–6.
[39] Huang J, Cai J, Wang J, Zhu X, Huang L, Yang S, et al. Efficient production of butyric acid from Jerusalem artichoke by immobilized *Clostridium tyrobutyricum* in a fibrous-bed bioreactor. Bioresour Technol 2011;102:3923–6.
[40] Himmel M, Ding S, Johnson D, Adney W, Nimlos M, Brady J, et al. Biomass recalcitrance: engineering plants and enzymes for biofuel production. Sci Mag 2007;315(5813):804–7.
[41] Eggeman T, Elander R. Process and economic analysis of pretreatment technologies. Bioresour Technol 2005;96:2019–25.
[42] Agbor V, Cicek N, Sparling R, Berlin A, Levin D. Biomass pretreatment: fundamentals toward application. Biotechnol Adv 2011;29:675–85.
[43] Kumar P, Barrett D, Delwiche M, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficicent hydrolysis and biofuel production. Ind Eng Chem Res 2009;48:3713–29.
[44] Chen W, Tsai C, Lin C, Tsai P, Hwang W. Pilot-scale study on the acid-catalyzed steam explosion of rice straw using a continuous pretreatment system. Bioresour Technol 2013;128:297–304.
[45] Murnen H, Balan V, Chundawat S, Bals B, Sousa L, Dale B. Optimization of Ammonia Fiber Expansion (AFEX) pretreatment and enzymatic hydrolysis of *Miscanthus x giganteus* to fermentable sugars. Biotechnol Prog 2007;23 (4):846–50.
[46] Hendriks A, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 2009;100:10–8.
[47] Kim K, Hong J. Supercritical CO_2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. Bioresour Technol 2001;77:139–44.
[48] Banerjee S, Sen R, Pandey RA, Chakrabarti T, Satpute D, Giri B, et al. Evaluation of wet air oxidation as a pretreatment strategy for bioethanol production from rice husk and process optimization. Biomass Bioenergy 2009;33:1680–6.
[49] Banerjee S, Sen R, Morone A, Chakrabarti T, Pandey RA, Mudliar S. Improved wet air oxidation pretreatment for enhanced enzymatic hydrolysis of rice husk for bioethanol production. Dyn Biochem Process Biotechnol Mol Biol 2012;6(2):43–5.
[50] Carvalheiro F, Duarte L, Gírio F. Hemicellulose biorefineries: a review on biomass pretreatment. J Sci Ind Res 2008;67:849–64.
[51] Zhao Y, Wang Y, Zhu JY, Ragauskas A, Deng Y. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. Biotechnol Bioeng 2008;99(6):1320–8.
[52] Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol 2009;82:815–27.
[53] Euphrosine-Moy V, Lasry T, Bes S, Molinier J, Mathieu J. Degradation of poplar lignin with ozone. Ozone Sci Eng 1991;13(2):239–48.
[54] Ungurean M, Fiţigău F, Paul C, Ursoiu A, Peter F. Ionic liquid pretreatment and enzymatic hydrolysis of wood biomass. World Acad Sci Eng Technol 2011;76:387–91.
[55] Volynets B, Dahman Y. Assessment of pretreatments and enzymatic hydrolysis of wheat straw as a sugar source for bioprocess industry. Int J Energy Environ 2011;2(3):427–46.
[56] Banerjee G, Car S, Scott-Craig J, Hodge D, Walton J. Alkaline peroxide pretreatment of corn stover: effects of biomass, peroxide, and enzyme loading and composition on yields of yield of glucose and xylose. Biotechnol Biofuels 2011;4:16.
[57] Yu J, Jibin Z, He J, Liu Z, Yu Z. Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull. Bioresour Technol 2009;100:903–8.
[58] Bouton J. Molecular breeding of switchgrass for use as a biofuel crop. Curr Opin Genet Dev 2007;17:553–8.
[59] Fu C, Mielenz J, Xiaoa X, Gea Y, Hamilton C, Rodriguez M, et al. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. Proc Natl Acad Sci USA 2010;108:3803–8.
[60] Sanderson K. Lignocellulose – a chewy problem. Nat Outlook: Biofuels 2011;474(7352):12–4.
[61] Chundawat S, Beckham G, Himmel M, Dale B. Deconstruction of lignocellulosic biomass to fuels and chemicals. Annu Rev Chem Biomol Eng 2011;2:121–45.
[62] Zhu L, O'Dwyer J, Chang V, Granda C, Holtzapple M. Structural features affecting biomass enzymatic digestibility. Bioresour Technol 2008;99:3817–28.
[63] Kim S, Holtzapple M. Effect of structural features on enzyme digestibility of corn stover. Bioresour Technol 2006;97:583–91.
[64] Adsul M, Singhvi M, Gaikaiwari S, Gokhale D. Development of biocatalysts for production of commodity chemicals from lignocellulosic biomass. Bioresour Technol 2011;102:4304–12.
[65] Menon V, Rao M. Trends in bioconversion of lignocellulose: biofuels, platform chemicals and biorefinery concept. Prog Energy Combust Sci 2012;38:522–50.
[66] MacLellan J. Strategies to enhance enzymatic hydrolysis of cellulose in lignocellulosic biomass. MMG 445 Basic Biotechnol 2010;6:31–5.
[67] Zhang Y, Fu E, Liang J. Effect of ultrasonic waves on the saccharification processes of lignocelluloses. Chem Eng Technol 2008;31(10):1510–5.
[68] Yachmenev V, Condon B, Klasson T, Lambert A. Acceleration of the enzymatic hydrolysis of corn stover and sugar cane bagasse celluloses by low intensity uniform ultrasound. J Biobased Mater Bio 2009;3:25–31.
[69] Li C, Yoshimoto M, Oqata H, Tsukuda N, Fukunaga K, Nakao K. Effects of ultrasonic intensity and reactor scale on kinetics of enzymatic saccharification of various waste papers in continuously irradiated stirred tanks. Ultrason Sonochem 2005;12(5):373–84.
[70] Zhang Y, Liu Y, Xu J, Yuan Z, Qi W, Zhuang X, et al. High solid and low enzyme loading based saccharification of agricultural biomass. Bioresources 2012;7(1):345–53.
[71] Genencor launches three new Accellerase® enzyme products for cellulosic ethanol process development. 〈http://biosciences.dupont.com/media/news-archive/news/2009/genencor-launches-three-new-acceleraser-enzyme-products-for-cellulosic-ethanol-process-development/〉.
[72] Advanced biofuels becoming reality with Novozymes' new enzyme technology. 〈http://www.novozymes.com/en/news/news-archive/Pages/Advanced-biofuels-becoming-reality-with-Novozymes-new-enzyme-technology.aspx〉; 2012.
[73] Codexis announces next generation Codexyme® cellulose enzymes with leading performance. 〈http://ir.codexis.com/phoenix.zhtml?c=208899&p=irol-newsArticle&ID=1794687&highlight=〉.
[74] Dürre P. Fermentative butanol production – bulk chemical and biofuel. Ann N Y Acad Sci 2008;1125:353–62.
[75] Green E. Fermentative production of butanol – the industrial perspective. Curr Opin Biotechnol 2011;22:1–7.
[76] Ezeji T, Qureshi N, Blaschek H. Butanol fermentation research: upstream and downstream manipulations. Chem Rec 2004;4:305–14.
[77] Qureshi N, Saha B, Cotta M. Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part II – fed-batch fermentation. Biomass Bioenergy 2008;32:176–83.
[78] Thirmal C, Dahman Y. Different physical and chemical pretreatments of wheat straw for enhanced biobutanol production in simultaneous saccharification and fermentation. Int J Energy Environ 2011;2(4):615–26.
[79] Carere C, Sparling R, Cicek N, Levin D. Third generation biofuels via direct cellulose fermentation. Int J Mol Sci 2008;9:1342–60.
[80] Higashide W, Li Y, Yang Y, Liao J. Metabolic engineering of *Clostridium cellulolyticum* for production of isobutanol from cellulose. Appl Environ Microbiol 2011;77(8):2727–33.
[81] Ezeji T, Qureshi N, Blaschek H. Continuous butanol fermentation and feed starch retrogradation: butanol fermentation sustainability using *Clostridium beijerinckii* BA101. J Biotechnol 2005;115:179–87.
[82] Liew ST, Arbakariya A, Rosfarizan M, Raha AR. Production of solvent (acetone–butanol–ethanol) in continuous fermentation by *Clostridium saccharobutylicum* DSM 13864 using gelatinised sago starch as a carbon source. Malays J Microbiol 2006;2(2):42–50.
[83] Ramey D. Continuous two stage, dual path anaerobic fermentation of butanol and other organic solvents using two different strains of bacteria. United States Patent. Patent no. 5,753,474; 1998.
[84] Jiang L, Wang J, Liang S, Cai J, Xu Z, Cen P, et al. Enhanced butyric acid tolerance and bioproduction by *Clostridium tyrobutyricum* immobilized in a fibrous bed bioreactor. Biotechnol Bioeng 2011;108(1):31–40.
[85] Gheshlaghi R, Scharer JM, Moo-Young M, Chou CP. Metabolic pathways of clostridia for producing butanol. Biotechnol Adv 2009;27:764–81.
[86] Zheng Y, Li L, Xian M, Ma Y, Yang J, Xu X, et al. Problems with the microbial production of butanol. J Ind Microbiol Biotechnol 2009;36:1127–38.
[87] Lütke-Eversloh T, Bahl H. Metabolic engineering of *Clostridium acetobutylicum:* recent advances to improve butanol production. Curr Opin Biotechnol 2011;22:634–47.
[88] Shen C, Lan E, Dekishima Y, Baez A, Cho K, Liao J. Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. Appl Environ Microbiol 2011;77(9):2905–15.

[89] Huang H, Liu H, Gan Y. Genetic modification of critical enzymes and involved genes in butanol biosynthesis from biomass. Biotechnol Adv 2010;28:651–7.
[90] Knoshaug E, Zhang M. Butanol tolerance in selection of microorganisms. Appl Biochem Biotechnol 2009;153:13–20.
[91] Dunlop MJ. Engineering microbes for next-generation biofuels. Biotechnol Biofuels 2011;4:32.
[92] Rühl J, Schmid A, Blank L. Selected *Pseudomonas putida* strains able to grow in the presence of high butanol concentration. Appl Environ Microbiol 2009;75(13):4653–6.
[93] Atsumi S, Cann A, Connor M, Shen C, Smith K, Brynildsen M, et al. Metabolic engineering of *Escherichia coli* for 1-butanol production. Metab Eng 2008;10:305–11.
[94] Kataoka N, Tajima T, Kato J, Rachadech W, Vangnai AS. Development of butanol-tolerant *Bacillus subtilis* strain GRSW2-B1 as a potential bioproduction host. AMB Express 2011;1:10.
[95] Liu S, Qureshi N. How microbes tolerate ethanol and butanol? New Biotechnol 2009;26(3/4):117–20.
[96] Nielson D, Leonard E, Yoon S, Tseng H, Yuan C, Prather K. Engineering alternative butanol production platforms in heterologous bacteria. Metab Eng 2009;11:262–73.
[97] Wu D, Wang Q, Assary R, Broadbelt L, Krilov G. A computational approach to design and evaluate enzymatic reaction pathways: application to 1-butanol production from pyruvate. J Chem Inf Model 2011;51:1634–47.
[98] Ranganathan S, Maranas C. Microbial 1-butanol production: identification of production routes and *in silico* engineering interventions. Biotechnol J 2010;5:716–25.
[99] Haus S, Jabbari S, Millat T, Janssen H, Fischer R, Bahl H, et al. A systems biology approach to investigate the effect of pH-induced gene regulation on solvent production by *Clostridium acetobutylicum* in continuous culture. BMC Syst Biol 2011;5:10.
[100] Zhu L, Zhu Y, Zhang Y, Li Y. Engineering the robustness of industrial microbes through synthetic biology. Trends Microbiol 2012;20(2):94–101.
[101] Tomas CA, Welker NE, Papoutsakis ET. Overexpression of *groESL* in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. Appl Environ Microbiol 2003;69:4951–65.
[102] Reyes L, Almario M, Kao K. Genomic library screens for genes involved in butanol tolerance in *Escherichia coli*. PLoS One 2011;6(3):1–9 (Available at: ⟨www.plosone.org⟩).
[103] Oudshoorn A, van der Wielen LAM, Straathof AJJ. Assessment of options for selective 1-butanol recovery from aqueous solution. Ind Eng Chem Res 2009;48:7325–36.
[104] Ezeji T, Qureshi N, Blaschek H. Bioproduction of butanol from biomass: from genes to bioreactors. Curr Opin Biotechnol 2007;18:220–7.
[105] Kamiński W, Tomczak E, Górak A. Biobutanol – production and purification methods. Ecol Chem Eng S 2011;18(1):31–7.
[106] Vane LM. A review of pervaporation for product recovery from biomass fermentation processes. J Chem Technol Biotechnol 2005;80:603–9.
[107] Groot WJ, van der Lans RGJM, Luyben KChAM. Technologies for butanol recovery integrated with fermentation. Process Biochem 1992;27(2):61–75.
[108] CRC. Handbook of chemistry and physics. Boca Raton (FL): CRC Press; 2004.
[109] Qureshi N, Hughes S, Maddox IS, Cotta MA. Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst Eng 2005;27:215–22.
[110] Tashiro Y, Takeda K, Kobayashi G, Sonomoto K. High production of acetone–butanol–ethanol with high cell density culture by cell-recycling and bleeding. J Biotechnol 2005;120:197–206.
[111] Jovanovic J, Rebrov EV, Nijhuis TA, Kreutzer MT, Hessel V, Schouten JC. Liquid–liquid flow in capillary microreactor: hydrodynamic flow patterns and extraction performance. Ind Eng Chem Res 2012;51(2):1015–26.
[112] Ezeji T, Qureshi N, Blaschek H. Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J Microbiol Biotechnol 2003;19:595–603.
[113] Ezeji T, Qureshi N, Blaschek H. Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 2004;63:653–8.
[114] Qureshi N, Blaschek HP. Recovery of butanol from fermentation broth by gas stripping. Renew Energy 2001;22(4):557–64.
[115] Chen C., Fawcett A., Posner A., Raviv T. Butanol by two stage fermentation. Senior design reports (CBE). University of Pennsylvania. Available at: ⟨http://repository.upenn.edu⟩; 2009.
[116] Ponce-Ortega JM, Al-Thubaiti MM, El-Halwagi MM. Process Intensification: new understanding and systematic approach. Chem Eng Process 2012;53:63–75.
[117] Ikwebe J, Harvey AP. Intensification of bioethanol production by simultaneous saccharification and fermentation (SSF) in an oscillatory baffled reactor (OBR). Bioenergy Technol 2011:381–8.
[118] Kopke M, Held C, Hujer S, Liesegang H, Wiezer A, Wollherr A, et al. *Clostridium ljungdahlii* represents a microbial production platform based on syngas. Proc Natl Acad Sci 2010;107(29):13087–92.
[119] Nimcevic D, Gapes R. The acetone–butanol fermentation in pilot plant and pre-industrial scale. J Mol Microbiol Biotechnol 2000;2(1):15–20.
[120] Butamax Technology. ⟨http://www.butamax.com⟩.
[121] Gevo Science & Technology. ⟨http://www.gevo.com/about/our-business/our-science-and-technology/⟩.
[122] Cathay Industrial Biotech. Nat Biotechnol 2012;30(6). ⟨http://www.cathaybiotech.com/en/docs/media-060112.pdf⟩.
[123] Cobalt Technologies successfully scales commercial n-butanol production. ⟨http://www.cobalttech.com/pdf/cobaltfacts.pdf⟩; 2013.
[124] Companies working on producing biobutanol. ⟨http://www.biobutanol.com/Biobutanol-Producers-Gevo-Butamax-Cobalt.html⟩.
[125] Syntec Biofuel Technology. ⟨http://www.syntecbiofuel.com/technology.php⟩.
[126] Biobutanol Technology – Butalco. ⟨http://www.butalco.com/Technology/BiobutanolTechnology/biobutanol_technology.html⟩.